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Title: Changes in 10-year cardiovascular risk and behavioral risk factors in men in Crete, Greece, since the Seven Countries Study (1960-1997)

Running Head: CVD risk and behavioral risk factors changes

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Abstract

Aim: The Seven Countries Study showed that in the 1960s, participants in the island of Crete, Greece, had among the lowest cardiovascular (CVD) mortality rates in the world, but in recent years CVD rates in Crete have increased. This study aimed to assess changes in CVD-risk occurring since the Seven Countries Study.

Subject and Methods: Secondary analysis was performed in two cohorts of men (aged 40-60 years) in Crete-Greece, in 1960 (Seven Countries Study participants) and 1997 (random sample of men of the same age and residence areas). The HeartScore was used to estimate the (i) 10-year CVD-risk and (ii) 10-year CVD-risk based on body mass index (BMI), of 627 (1960 cohort) and 93 (1997 cohort) Cretan men.

Results: Between 1960 and 1997, 10-year CVD-risk (3.4 vs. 4.7%, $P<0.001$) and risk based on BMI (1.6 vs. 3.0%, $P<0.001$) increased by 38% and 88%, respectively. During this period, the proportion of overweight/obese participants (21.8 vs. 83.9%, $P<0.001$) and participants with hypercholesterolemia (51.5 vs. 81.7%, $P<0.001$), as well as physical inactivity (37.3 vs. 56.0%, $P=0.001$) increased. The prevalence of high and very high CVD-risk ($\geq 5\%$) almost doubled (+90%), mainly due to concomitant increases in total cholesterol levels (+84%, $P<0.001$) during this period. CVD-risk increased between 1960 and 1997 in this population of Cretan men.

Conclusions: Appropriate public health initiatives are needed to promote the traditional, health-promoting lifestyle of men in Crete observed in the 1960s in the Seven Countries Study.

Keywords

10-year Cardiovascular Risk; Secular Changes; Seven Countries Study; Nutrient Intake; Cretan Men

Introduction

Cardiovascular (CVD) diseases, as a group of disorders of the heart function and arteries, are the main cause of mortality in developed countries, while more than 80% of CVD-related deaths occur in low- and middle-income countries (WHO 2011).

The Seven Countries Study is among the longest and most important studies to have tested the diet-cholesterol-coronary heart disease (CHD) hypothesis (Keys et al. 1966). In this study, Crete (Greece) was found to be the region with the lowest CHD prevalence and incidence due to low serum cholesterol concentrations, high physical activity and a diet that was low in saturated fat and high in monounsaturated fat and plant foods (the traditional Mediterranean diet) (Kafatos et al. 1997). However, during the 2000's Greek men and women aged 50+ years had the fourth and third highest prevalence of CVD respectively, compared to other Europeans (Vassilaki et al. 2014), while only 2.9% (4 million) of US adults had a 10-year CHD risk of >20% (high risk) (Ford et al. 2004).

The aim of the present study was to assess the 10-year CVD risk in two cohorts of middle-aged (40-60 years) men in Crete, Greece, in 1960 (Seven Countries Study participants) and 1997 (random sample of men of the same age and residence areas as the Seven Countries study participants) using the HeartScore system, a tool which estimates the absolute risk of developing CVD, as well as examine changes in CVD risk and behavioral risk factors during this 37-year period.

Methods

Study Population

A secondary analysis of two cohorts of men was conducted for the purposes of the current study: men (aged 40-60 years) from Crete, Greece, participating in the Seven Countries Study in 1960 (n=686) (<http://www.sevencountriesstudy.com/about-the-study/countries/>) and men of the same age, randomly chosen from the male residents of the Seven Countries Study participating Cretan villages in 1997 (n=112) (Hatzis et al. 2013; Kafatos et al. 1997; Keys et al. 1966). Thirty men (4.4%) of the 1960 cohort and 12 (10.7%) from the 1997 cohort were excluded from the current analysis because they had already been diagnosed with CVD (e.g. angina pectoris, coronary artery disease, myocardial infarction, ischemic stroke or diabetes mellitus). Additionally, 29 and 7 men, respectively, were excluded due to missing data. The total sample included in the current study was therefore 627 (1960 cohort) and 93 (1997 cohort) men. Follow-ups were carried out in 1970 (for the 1960 cohort) and 2010 (for the 1997 cohort), during which 593/627 men from the 1960 cohort and 78/93 men from the 1997 cohort were assessed for 10-year CVD risk and 10-year CVD mortality risk. It is mentioned that the experimental protocols and the process for obtaining informed consent were approved by the Ethics Committee of the University of Crete (Hatzis et al. 2013; Keys et al. 1966; Schiele et al. 2000).

Data collection

In the final sample, the 10-year CVD risk was calculated using the HeartScore system (European Society's 10-year Systematic Coronary Risk Estimation - SCORE, <http://www.heartscore.org>), using systolic blood

pressure, total serum cholesterol levels and self-reported smoking status (Panagiotakos et al. 2007). A 10-year CVD risk of $\geq 5\%$ was considered to be high or very high (Piepoli et al. 2016). In addition, the 10-year CVD risk based on body mass index (BMI) was estimated using objective body weight (in kg) and height (in meters) measurements. All measurements were conducted using pre-specified standard operating procedures (Kafatos et al. 1997; Vardavas et al. 2009).

Physical inactivity was presented as informational characteristic. The classification of physical activity was determined through the question of “*Physical activity at work*” for the cohort of 1960 in three categories: “*bedridden or sedentary & light*”, “*moderate*” and “*very active or heavy & very heavy*”. For the cohort of 1997, the classification in the respective three categories was determined through a physical activity questionnaire assessing the PAL (Physical Activity Level) index, which included questions about the type and intensity of physical activity in the context of general health habits (work and leisure) per week (number of hours) and per year (number of months). More details about the recruitment of the two cohorts of men and data collection protocols are described elsewhere (Hatzis et al. 2013; Kafatos et al. 1997; Keys et al. 1966; Vardavas et al. 2009).

A 7-day weighed food inventory and a previously validated food frequency questionnaire, requesting participants to report their food intake during the previous month, were used to assess average daily energy, nutrient and food intake for men of the 1960 and 1997 cohorts, respectively (Kafatos et al. 1997; Kafatos et al. 1991; Kromhout et al. 1989; Kromhout et al. 2002). Data from the 1960 dietary assessment were coded by the same dietitian in 1985-1986, using pre-specified protocols and the mean consumption of foods, categorized into 16 food groups, was estimated (Hatzis et al. 2013). For the energy and nutrient analyses of these data, two dietitians collected foods and ingredients reported in the inventories from the local markets in 1987. These foods were shipped on dry ice to the Laboratory of Human Nutrition, Wageningen University, the Netherlands, where they were homogenized and stored (-20°C) until chemical analyses for nutrient calculation were carried out. Total fat content was determined using the method described by Osborne and Voogt (Osborne and Voogt 1978). Total energy was calculated by summing total protein, fat and carbohydrate content. Dietary fiber content was determined enzymatically (Kromhout et al. 2001; William 1985). Data from the 1997 dietary assessment were coded and analyzed using the food composition database Greek Diet, which was created by the Preventive Medicine and Nutrition Clinic, Faculty of Social Medicine, University of Crete in 1990 and was upgraded in 2000 (Kafatos et al. 2000). For the current report, food group consumption categories for both cohorts contain the same foods, except in the case of fish, where the 1997 cohort category also contains seafood.

Statistical analysis

Data were analyzed using the SPSS software (IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp). Analysis of covariance was used to assess changes in different CVD risk factors between 1960 and 1997, with age as a covariate (in addition to body weight and smoking when changes in blood pressure and total serum cholesterol levels were assessed). The Mann Whitney (and/or one sample student t test) and Chi-Square tests were used to assess changes in continuous and categorical 10-year CVD risk

factors, respectively. The CVD incidence rate in follow-up periods was estimated using Poisson distribution according to time to follow-up (interval between each study's commencement and follow-up) or death (per 10,000 person-years), whichever occurred first.

Results

Between 1960 and 1997, significant increases were observed in body weight (+16.4kg, $P<0.001$) and the proportion of overweight/obese participants (+285%, $P<0.001$), as well as in total cholesterol levels (+19.6mg/dl, $P<0.001$) (Table 1). At the same time, systolic and diastolic blood pressure decreased, but the proportion of participants with hypertension (-4%, $P=0.790$) and smoking prevalence (-15%, $P=0.128$) did not change significantly. In contrast, prevalence of physical inactivity increased by +50% ($P=0.001$). A significant increase (+38%, $P<0.001$) in 10-year CVD risk between the two time periods was also observed, while the prevalence of men with high levels ($\geq 5\%$) of CVD risk almost doubled (+90%, $P<0.001$). This increase was mainly attributed (for men with CVD risk $>1\%$) to concomitant increases in total cholesterol levels (+84%, $P<0.001$). The 10-year CVD risk based on BMI also increased by 88% (from 1.6% in 1960 to 3.0% in 1997, $P<0.001$) (Table 1).

→ TABLE 1

Total mean daily energy intake did not change between 1960 and 1997 (Table 2). Several unfavorable changes in nutrient intakes occurred during this period, including increases in the mean daily intakes of saturated fat (+3.2g, $P=0.036$) and dietary cholesterol (+60mg, $P<0.001$), and decreases in the intakes of monounsaturated fat (-29.7g, $P<0.001$) and dietary fiber (-17g, $P<0.001$). As far as foods are concerned, unfavorable changes between 1960 and 1997 were observed in the mean daily consumption of meat (+56g, $P<0.001$), olive oil (-56g, $P<0.001$) and alcohol (+33g, $P<0.001$). In contrast, consumption of cereals (+49g, $P<0.001$), fruits and vegetables (+107g, $P=0.046$), fish (+10g, $P<0.001$) and legumes (+19g, $P<0.001$) increased during this 37-year period (Table 3).

→ TABLES 2, 3

Three (of 593) participants of the 1960 cohort (mean CVD risk, 7.7) and one (of 78) participant of the 1997 cohort (CVD risk, 7.0) died from CVD causes by the follow-ups of 1970 and 2010, respectively. The respective CVD incidence rates (per 10,000 person-years) were 5.2 (95% CI, 1.1-15.1) and 10.1 (95% CI, 0.3-56.3) (results not shown in table or figure).

Discussion

In this sample of middle-aged Cretan men, a significant increase in 10-year CVD risk was observed from 1960, when the Seven Countries Study commenced, to 1997. In addition, the prevalence of men with high and very high 10-year CVD risk almost doubled in this 37-year period, while the factors contributing to the observed 10-year CVD risk increase concomitantly changed. During this period, prevalence of overweight/obesity, physical inactivity and hypercholesterolemia increased, while several unfavorable dietary changes occurred. These findings suggest that secular changes in these men's behavioral risk factors

for CVD, as well as lifestyle habits, such as diet and physical inactivity, might have played a role in the observed increase in CVD risk.

The proportion of overweight/obese participants increased almost fourfold in the examined 37-year period, with a concomitant, almost twofold, increase in 10-year CVD risk based on BMI. This important increase in the prevalence of obesity and CVD risk has been suggested to stem from changes in dietary habits in the Cretan population. As previously reported, the traditional Mediterranean diet of Crete has gradually been abandoned by the Seven Countries Study's participants, who, over the decades, increased their consumption of meat and cheese, as well as their intake of saturated fat, and decreased their consumption of bread and intake of dietary fiber (Kafatos et al. 1997). This emphasizes the need for the development of structured population dietary guidelines, which are currently lacking in Greece, that specifically advocate the health-promoting benefits of the traditional Mediterranean diet of Crete (Moschandreas and Kafatos 1999). However, an increase in the consumption of cereals, fruits and vegetables, fish and legumes was observed in these 37 years, which constitutes a positive change in men's dietary habits. This change might be attributed to increased food availability for these participants living in rural areas of Crete, due to increases in productivity, improved agricultural practice, greater food diversity and less seasonal dependence. Food availability has also increased as a consequence of rising income levels and falling food prices (Kearney 2010).

In addition to the potential role that changes in dietary habits had in the increase in obesity prevalence in the examined 37-year period, the current study showed that the aforementioned increase in the prevalence of obesity has been accompanied by a concomitant increase in physical inactivity prevalence. In particular, the proportion of physically inactive participants and those who engaged in light or moderate, as opposed to vigorous, activity increased from 37.3% in 1960 to 56% in 1997. This finding agrees with an earlier report in this sample of men whose occupations were mainly agricultural and therefore were likely to engage in vigorous physical activities in the 1960s (Vardavas et al. 2009; Vardavas et al. 2010). This increase in physical inactivity is potentially the result of the use of technologically-advanced farm machinery over the decades, which has reduced the need for human physical labor. Regarding the other lifestyle habit assessed in the current study, smoking prevalence decreased by 15% in this 37-year period and the 10-year CVD risk attributable to smoking also decreased by 28%. The observed reduction in smoking prevalence most likely stems from higher levels of obtained knowledge of smoking's harmful health effects over the decades, and among the 1997 cohort, compared to participants in 1960, as health warnings have been suggested by an earlier study to reduce smoking more, compared to the taxation of tobacco products (Alpert et al. 2014).

The significant increase in the proportion of participants with hypercholesterolemia between 1960 and 1997 in the current analysis, as well as the concomitant increase in the 10-year CVD risk attributable to cholesterol levels might be the result of the unfavorable dietary changes, as well as the increase in physical inactivity observed during this period. High levels of total cholesterol have previously been directly related to these two lifestyle behaviours (Enger et al. 1977; Mensink and Katan 1990; Sunami et al. 1999). The finding that levels of both systolic and diastolic blood pressure decreased in these 37 years is potentially

the result of global improvements in the treatment and primary care prevention of hypertension (Vardavas et al. 2010). As a consequence, it was also noted that the 10-year CVD risk attributed to hypertension also decreased in this period.

Study limitations and strengths

The study's epidemiological sampling approach and methodology allow our findings to be generalized to the farming population of Crete (Hatzis et al. 2013; Kafatos et al. 1997; Keys et al. 1966; Moschandreas and Kafatos 1999; Vardavas et al. 2009; Vardavas et al. 2010). However, the low sample size of the 1997 cohort reduces the power of the results, since only 112 of 326 men (34%) whose ages were between 40 and 60 years and were residing in the same villages as their Seven Countries Study counterparts (1960 cohort), and who qualified for participation in the survey, agreed to be examined (Hatzis et al. 2013). Moreover, there is a possibility that laboratory differences resulting from the availability of newer equipment have affected the assessment of blood cholesterol levels, even though the methods to assess this biochemical index have remained the same over the years.

Conclusions

The current findings indicate that unfavorable changes have occurred in CVD risk and behavioral risk factors for CVD in the Cretan rural middle-aged population in a 37-year period. This suggests that there is an urgent need for appropriate public health and nutrition education initiatives to promote the traditional lifestyle of men in Crete, as documented in the 1960s by the Seven Countries Study.

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Disclosure of potential conflicts of interest:

- **Conflict of Interest: The authors declare that they have no conflict of interest.**
- **Funding: This research received no specific grant from any funding agency, commercial or not-for-profit sectors.**

Compliance with Ethical Standards

Research involving Human Participants

Ethical approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the Ethics Committee of the University of Crete and with 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent: Informed consent was obtained from all individual participants included in the study.

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Table 1. Risk factors for cardiovascular disease (CVD) and 10-year CVD risk in two cohorts of men in Crete, Greece, 1960-1997

Risk factors			Cohorts		<i>Δ</i> -change	P-value
			1960 (n=627)	1997 (n=93)		
Body weight,kg	<i>mean±s.e.</i>		63.0±0.4 ^a	79.4±1.0	+16.4	<0.001
Body Mass Index (BMI), kg/m²	<i>mean±s.e.</i>		22.8±0.1	28.1±0.3	+5.3	<0.001
<i>Overweight, Obese</i>	<i>n (%)</i>		136 (21.8) ^b	78 (83.9)	+285%	<0.001
Systolic blood pressure, mm Hg	<i>mean±s.e.</i>		137.3±0.7	123.7±2.1	-13.6	<0.001
Diastolic blood pressure, mm Hg	<i>mean±s.e.</i>		82.0±0.4	78.8±1.2	-3.2	0.018
<i>High blood pressure (>140/90 mm Hg)</i>	<i>n (%)</i>		211 (33.7)	30 (32.3)	-4%	0.790
Total cholesterol,mg/dl	<i>mean±s.e.</i>		208.6±1.7	228.2±4.9	+19.6	<0.001
<i>Hypercholesterolemia (>200 mg/dl)</i>	<i>n (%)</i>		323 (51.5)	76 (81.7)	+59%	<0.001
Physical inactivity (bedridden, sedentary, light, moderate)	<i>n (%)</i>		234 (37.3)	47 (56.0)	+50%	0.001
Smoking (current smokers)	<i>n (%)</i>		356 (56.8)	45 (48.4)	-15%	0.128
10-year CVD-risk^d	<i>mean [median] risk as %</i>		3.4 [3.0] ^c	4.7 [4.0]	+38%	<0.001
<i>High risk (≥5%)</i>	<i>n (%)</i>		142 (22.6)	40(43.0)	+90%	<0.001
10-year CVD-risk attributed to ^e:	Smoking	<i>mean [median] risk as %</i>	53.7 [69.0]	38.4 [21.0]	-28%	<0.001
	Systolic blood pressure	<i>mean [median] risk as %</i>	19.0 [0.0]	11.2 [0.0]	-41%	0.031
	Total cholesterol	<i>mean [median] risk as %</i>	27.3 [10.0]	50.3 [36.5]	+84%	<0.001
10-year CVD-risk based on BMI^d	<i>mean [median] risk as %</i>		1.6 [1.0]	3.0 [3.0]	+88%	<0.001

^aAnalysis of covariance with age as covariate (body weight and smoking were used as additional covariates in blood pressure and total cholesterol analysis).

^b Chi-square tests.

^c Mann Whitney tests.

^d The ‘10-year CVD risk’ is based on three parameters, i.e. smoking, systolic blood pressure and total cholesterol, and the ‘10-year CVD risk based on BMI’ is based on body weight, height and smoking.

^e Estimations refer to participants with risk ≥1% on each of three parameters (644 from 720 participants or 89.4%).

Table 2. Mean daily energy and nutrient intake in two cohorts of men in Crete, Greece, 1960-1997

Nutrients	Cohorts		Δ -change	<i>P</i> -value ^b
	1960 ^a	1997		
	(n=31)	(n=85)		
	Meanintake (SD)			
Energy (kcal)	2820	2831 (900)	-11	0.908
Protein (% total energy intake)	12.5	12.7 (2.8)	+0.2	0.506
Carbohydrates (% total energy intake)	43.0	43.9 (9.5)	+0.9	0.348
Total fat (% total energy intake)	41.9	35.9 (7.6)	-6.0	<0.001
Saturated fat (g)	28.0	31.2 (14.0)	+3.2	0.036
Saturated fat (% total energy intake)	8.9	9.8 (3.0)	+0.9	0.005
Monounsaturated fat (g)	84.1	54.4 (24.9)	-29.7	<0.001
Monounsaturated fat (% total energy intake)	26.8	17.4 (5.1)	-9.4	<0.001
Polyunsaturated fat (g)	13.4	12.6 (5.2)	-0.8	0.159
Polyunsaturated fat (% total energy intake)	4.4	4.0 (0.8)	-0.4	<0.001
Dietary fiber (g)	43	26.0 (9.6)	-17.0	<0.001
Cholesterol (mg)	211	271 (150)	+60	<0.001

Dietary assessment was conducted using a 7-day weighed food inventory (1960) and food frequency questionnaire requesting participants to report their food intake during the previous month (1997).

^a Adapted from bibliography(Hatzis et al. 2013; Kafatos et al. 1997; Kafatos et al. 1991; Vardavas et al. 2010). Standard deviations were not available for the 1960 cohort.

^b Differences over time were assessed using the one sample Student t-test.

Table 3. Mean daily food consumption in two cohorts of men in Crete, Greece, 1960-1997

Foods	Cohorts		Δ-change	P-value^b
	1960^a (n=31)	1997 (n=85)		
	Meanconsumption (SD)			
Bread (g/d)	380	230 (165)	-150	<0.001
Cereals (g/d)	30	79 (57)	+49	<0.001
Potatoes (g/d)	190	135 (75)	-55	<0.001
Legumes (g/d)	30	49 (43)	+19	<0.001
Vegetables and fruits (g/d)	655	762 (480)	+107	0.046
Meat (g/d)	35	91 (59)	+56	<0.001
Dairy products (g/d)	248	153 (147)	-95	<0.001
Fish (g/d)	18	28 (25)	+10	<0.001
Eggs (g/d)	25	26 (25)	+1	0.794
Olive oil (g/d)	95	39 (31)	-56	<0.001
Sugar, honey, sweets etc (g/d)	20	17 (15)	-3	0.138
Ethyl alcohol (g/d)	15	48 (46)	+33	<0.001

Dietary assessment was conducted using a 7-day weighed food inventory (1960) and food frequency questionnaire requesting participants to report their food intake during the previous month (1997). For the 1997 cohort, seafood was included in the fish food group.

^a Adapted from Adapted from bibliography(Hatzis et al. 2013; Kafatos et al. 1997; Kafatos et al. 1991; Vardavas et al. 2010). Standard deviations were not available for the 1960 cohort.

^b Differences over time were assessed using the one sample Student t-test.